Tree Physiology 33, 940–948
doi:10.1093/treephys/tpt067

Research paper

Foraging strategies in trees of different root morphology: the role of root lifespan

Thomas S. Adams, M. Luke McCormack and David M. Eissenstat[1]

Department of Ecosystem Science and Management and Intercollege Graduate Program in Ecology, Pennsylvania State University, University Park, PA 16802, USA; [1]Corresponding author (dme9@psu.edu)

Received April 12, 2013; accepted July 28, 2013; handling Editor Daniel Epron

Resource exploitation of patches is influenced not simply by the rate of root production in the patches but also by the lifespan of the roots inhabiting the patches. We examined the effect of sustained localized nitrogen (N) fertilization on root lifespan in four tree species that varied widely in root morphology and presumed foraging strategy. The study was conducted in a 12-year-old common garden in central Pennsylvania using a combination of data from minirhizotron and root in-growth cores. The two fine-root tree species, *Acer negundo* L. and *Populus tremuloides* Michx., exhibited significant increases in root lifespan with local N fertilization; no significant responses were observed in the two coarse-root tree species, *Sassafras albidum* Nutt. and *Liriodendron tulipifera* L. Across species, coarse-root tree species had longer median root lifespan than fine-root tree species. Localized N fertilization did not significantly increase the N concentration or the respiration of the roots growing in the N-rich patch. Our results suggest that some plant species appear to regulate the lifespan of different portions of their root system to improve resource acquisition while other species do not. Our results are discussed in the context of different strategies of foraging of nutrient patches in species of different root morphology.

Keywords: **localized nitrogen addition, minirhizotron, resource optimization, root efficiency, root foraging, root longevity.**

Introduction

The lifespan of roots is of broad interest in ecology. Because fine roots account for as much as one-third of global net primary productivity (Jackson et al. 1997), their lifespan is of major importance to carbon and nutrient cycles and is a key link to longer-term changes in soil organic matter and ecosystem carbon balance (Norby and Jackson 2000). Moreover, competition below ground occurs widely in plant communities and often dominates over competition above ground (Wilson 1988). Belowground competition may be largely associated with rapid exploitation of resource patches and control of the patches to the detriment of neighbors (Eissenstat and Caldwell 1988, Robinson et al. 1999). Although rapid root proliferation in nutrient-rich patches has been a major focus of research (Robinson 1996), much less is known about how patch exploitation is influenced by root longevity.

Nutrient acquisition is normally positively correlated with the total root length available for resource acquisition, but the total length at any point in time is not only a result of root production but also a result of root lifespan. Theoretically, root lifespan should be related to the lifetime efficiency of the root for resource uptake (Yanai et al. 1995, Eissenstat and Yanai 1997). Thus, root lifespan should be a function of the marginal benefits associated with resource acquisition relative to the marginal costs of maintaining the root that may also include evolutionary stable strategies that diminish the fitness of neighbors (e.g., O'Brien et al. 2007). Often the resource of interest is nitrogen (N), because it is commonly limiting for

plants (Vitousek and Howarth 1991), its mobility in soil (especially for nitrate) causes root competition to be likely and its availability in the soil matrix can vary by as much as an order of magnitude over small spatial scales (Jackson and Caldwell 1993). Nitrogen uptake is a costly process, which normally requires large energy expenditures associated with high protein turnover and assimilation (Bloom et al. 1992, Bouma et al. 1996). Therefore, root lifespan may shift in N-rich patches depending on the relative benefits associated with greater uptake compared with the relative costs associated with root construction, ion uptake and maintenance. The influence of the N patch on root longevity may also depend on the duration of the patch, the degree the patch differs from the bulk soil in N availability and the size of the patch (Fitter 1994).

Although few in number, studies of the lifespan of fine roots growing in localized N patches have shown mixed results. For example, Pregitzer et al. (1993) found that localized N fertilization increased root lifespan in a mixed Northern hardwood forest, whereas Bai et al. (2008) found that localized N fertilization decreased root lifespan of the grass, *Leymus chinensis*. Additionally, Hodge et al. (2009) found that in the grass *Lolium perenne*, the lifespan of roots inhabiting an N-rich patch can be extended or reduced in an unpredictable manner depending on the level of N enrichment. These variable results associated with the influence of localized N on root lifespan are exacerbated by methodological differences between studies, such as differences in the duration of the N application, the plant functional groups examined, the scale of the study systems and the determination of root lifespan. As a result, no clear picture has emerged concerning the role that localized N availability plays on the lifespan of fine roots.

In this study, we investigated the lifespan of fine roots growing in localized N-enriched patches that did not become depleted over time. The study was conducted in a common garden in central Pennsylvania using four Northeastern temperate tree species that varied widely in root morphology based on differences in root diameter and specific root length (SRL, root length–dry weight ratio). The root diameter and SRL are directly linked to costs of construction to produce the root length or surface area and have been linked to variation in both root proliferation (Eissenstat 1991) and root lifespan (Eissenstat et al. 2000, McCormack et al. 2012). For this study, we contrasted species with coarse-diameter first- and second-order roots (ordering classification follows a stream-based ordering system, Pregitzer et al. 2002) with those of fine diameter first- and second-order roots, because of the possible links between root construction costs and root lifespan. Specifically, we hypothesized that the fine-root species would proliferate roots more quickly and, to a greater extent, in nutrient-rich patches but would have shorter lived roots compared with those of the coarse-root species. We expected all species to increase root longevity in fertilized compared with unfertilized soil.

Materials and methods

All studies were conducted at a common garden planting located in central Pennsylvania, USA, at the Russell E. Larson Agricultural Research Center, Pennsylvania State University (40.8°N, 77.9°W). The common garden consists of 16 species of trees that were planted mostly in 1996 as 1-year-old liners in a randomized complete block design with eight blocks. Each species was planted in groups of six trees in a double row of three trees with a spacing of 3 m between trees within the row, 3 m between the double rows and 5 m spacing between the six-tree plots. We used four of the 16 trees species: two fine-root, high-SRL species (*Acer negundo* L., ACNE and *Populus tremuloides* Michx., POTR) and two coarse-root, low-SRL species (*Liriodendron tulipifera* L., LITU and *Sassafras albidum* Nutt., SAAL). All four of the species at this site were principally colonized by arbuscular mycorrhizas (Zadworny and Eissenstat 2011).

Soils were relatively fertile Hagerstown silt loam, well-drained, with a pH ranging from 6.1 to 6.5. Prior to planting the trees, the site was used as a grass hayfield. The entire area was fenced to keep out deer. Blocking was used to control variation in soil characteristics. Plants were obtained from local native-plant nurseries, except for *A. negundo* and *S. albidum*, which were collected from seedlings around State College, Pennsylvania. Understory vegetation was controlled within a half-meter of the trees using weed barrier cloth and gravel mulch, and sprayed with glyphosate to a distance of ~2 m from the trunk. Further from the trees, grass was mowed weekly or less frequently as needed. In June 2005, two 45-cm-long, 2.86 cm internal diameter (ID) clear acrylic minirhizotron observation tubes were installed at an angle of 30° from the vertical and a distance of 30 cm from the base of each study tree. This resulted in two tubes per tree spaced ~0.75 m apart. Tubes were installed in each of the eight blocks, resulting in 16 minirhizotron tubes per tree species. The minirhizotron tubes were equipped with a 12 cm length of 1.6 mm diameter irrigation tubing, which resulted in the irrigation tube running to a depth of 10 cm below the soil surface to enable localized fertilization (Eissenstat and Caldwell 1988). The portion of the minirhizotron tube above the soil surface was wrapped in black tape, stoppered and covered with a white aluminum can to minimize solar heating. One of the two tubes per tree was fertilized weekly throughout the growing season (roughly April to November), via the irrigation tube, with 10 ml of nutrient (NH_4NO_3) solution; the control tube was irrigated with 10 ml of deionized (DI) water.

The nutrient solution (10 ml of 98.1 mg NH_4NO_3 l^{-1}; three times 'available' soil solution N, see below) was added weekly to maintain a persistent localized N patch throughout the growing season of each year. The other tube of the pair received 10 ml of

DI water. In addition, 100 ml of DI water was also applied monthly to all tubes throughout the growing season, to flush any potential salt accumulation. Two years passed between the tube installation and first imaging session to allow conditions to equilibrate from the disturbance of the tube installation. Minirhizotron images were taken using a Bartz 1.125″ camera equipped with I-CAP version 4.01 imaging software (Bartz Technology, Carpinteria, CA, USA) at an interval of approximately every 3 weeks throughout the growing season for 3 years, 2007–2009. Initially, a single indexing hole was drilled in each tube to allow imaging from the upper viewing surface. After 1 year of imaging (i.e., in 2008), a second indexing hole was drilled on the lower viewing surface of the tube, allowing for twice the number of observations per tube. Root data were collected from the minirhizotron images using RootFly 1.8.35 (Wells and Birchfield, Clemson University, SC, USA). Roots were considered dead when a root was observed to have shriveled to approximately half the original diameter. As such, some roots may have been functionally dead before they were classified as dead in our analysis, which could potentially lead to overestimations of root lifespans. However, we expect this overestimation to have been minimal as a result of relatively active decomposition rates under the warm, mesic conditions of the common garden site. Additionally, because death was based on the original diameter of the observed roots, this process minimized the potential for species bias in determining root lifespan. New roots falling outside the measured diameter range of the first- and second-order roots of each species were excluded from analyses (McCormack et al. 2012), thereby controlling for encroachment of roots from neighboring species plots. Additionally, roots observed in the first imaging session were not included in the estimation of root lifespan because their birth date was unknown. The number of roots analyzed from this 3-year study were as follows: POTR $n = 564$, 337 from N-fertilized tubes and 227 from tubes receiving only water; ACNE $n = 346$, 191 from N-fertilized tubes and 155 from tubes receiving only water; LITU $n = 149$, 96 from N-fertilized tubes and 53 from tubes receiving only water; SAAL $n = 95$, 45 from N-fertilized tubes and 50 from tubes receiving only water.

To ascertain the level of localized N fertilization to be applied to the fertilized patches, we averaged the three highest values of soil solution N found in the common garden ($n = 32$). We averaged the highest soil solution N values found, under the assumption that plants would forage preferentially for the higher N. Soil solution N was determined using a saturated paste approach. Briefly, in July 2007, 25 g soil samples were collected to a 10 cm depth in two blocks for each of the 16 species at a distance of 50 cm from the trunk of the tree. Soil was moistened with water until the surface glistened and then centrifuged (10,000 rpm for 20 min). The supernatant was analyzed for nitrate and ammonium using a Lachat Quikchem 8500 autoanalyzer (Hach Co., Loveland, CO, USA). Using this approach, we determined that the available N at the common garden in naturally occurring high-N patches averaged 11.4 mg N l^{-1}. Previous studies on tree seedling growth on similar soil at a site adjacent to the common garden found low soil N values (0.13–0.15 mg nitrate (kg soil)$^{-1}$) and low seedling foliar N content, leading the investigators to conclude that N was limiting at the site (Harpster 2011).

In June 2007, root in-growth cores were installed by pounding a 7-cm ID steel tube into the ground to a depth of 30 cm. Soil was removed from the core, sieved of existing roots and returned to the hole. Five in-growth cores, corresponding to five N fertilization levels (0, 3, 10, 20 and 30 times soil solution N (11.4 mg N l^{-1})), were created in four blocks of three species (*A. negundo*, *P. tremuloides* and *L. tulipifera*), resulting in a total of 60 in-growth cores. *Sassafras albidum* was not used in the in-growth core portion of the experiment due to the low root densities observed for this species, which would have prevented the collection of adequate root sample for analyses. In-growth cores were marked with color coded 7.5-cm ID PVC pipe cut into 2.5-cm high rings, which also served as reservoirs for the fertilization solution. Each in-growth core received 100 ml of one of the five levels of N weekly, which allowed for soil saturation to ~30 cm. After ~3 months, a smaller 5 cm ID steel tube was pounded inside the existing core to a depth of 20 cm. Immediately after coring occurred, one to two small, intact first- and second-order root branches (where first-order roots are distal) were dissected from the total root pool, rinsed in DI water to remove any attached soil and analyzed for respiration using a Clark-type oxygen electrode (Hansatech Oxygraph, King's Lynn, UK). The use of intact first- and second-order root branches minimized root wounding and any resulting effects on root respiration. After the respiration measurement, roots were frozen, freeze dried, weighed to obtain dry mass and then ground with a mortar and pestle for nitrogen : carbon (N : C) analysis (Fisons EA 1108 CNS-O Analyzer, Fisons Instruments, Mt Pleasant, NJ, USA). Roots used to determine the SRL and root diameter by branching order were dug directly from the soil beneath the species of interest from four blocks in the spring of 2009. Roots were cleaned of soil with water, dissected to order and scanned on an Epson Perfection 4490 desk top scanner. The root length and diameter were then obtained from the scanned images using WinRhizo software (Regent Instruments, Quebec City, Quebec, Canada). After scanning, the roots were oven dried at 60 °C for 24 h and weighed. The SRL (in m g^{-1}) was calculated by dividing the length of the root sample by its dry mass.

Statistical analyses and root lifespan determination were conducted using SAS JMP 9.02 (SAS Institute, Inc., Cary, NC, USA). Log-rank tests were used to determine the significance of fertilization on root lifespan for each species (Figure 1). Cox proportional hazards tests (Cox 1972) were used to identify fine-root traits that had a significant effect on fine-root lifespan (Table 1). These traits included rooting depth, number of neighboring roots, season of birth and any resulting interactions with the N fertilization treatment. Rooting depth was determined by the depth of the

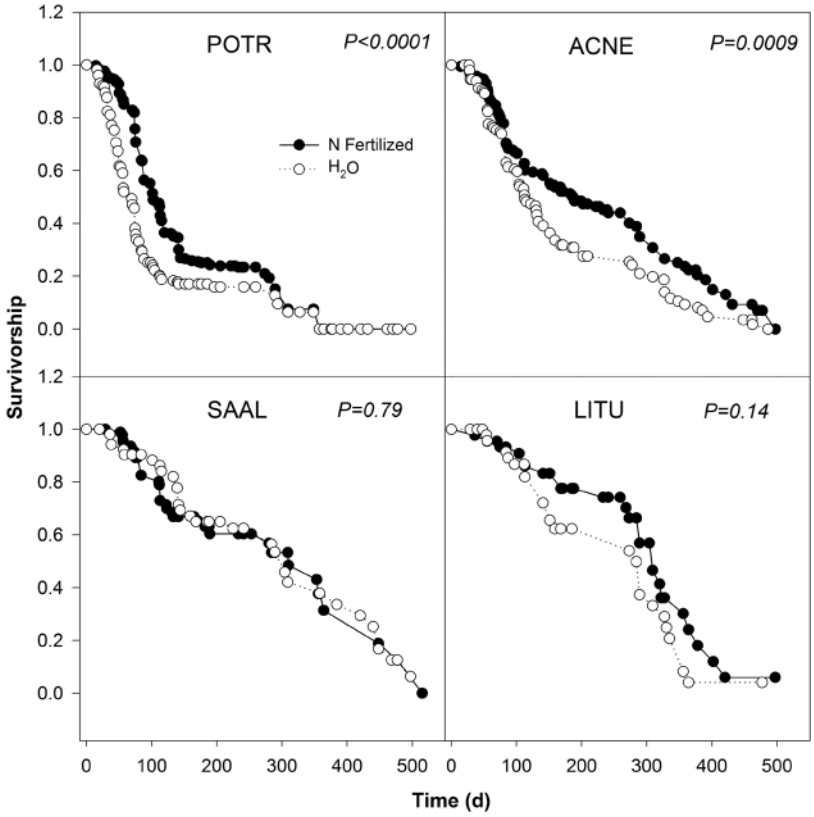

Figure 1. Survival probability curves of roots in localized patches of four tree species that varied widely in root morphology (*P* values indicate significance of fertilization effect for each species analyzed separately). Tree species were *A. negundo* (ACNE), *P. tremuloides* (POTR), *L. tulipifera* (LITU) and *S. albidum* (SAAL). The sustained N-fertilized patch treatment (N fertilized, closed circles with solid line) was three times background soil solution N, while the control patch (H_2O, open circles with dotted line) contained no additional N.

Table 1. Data table showing *P* values with Cox proportional hazard risk ratios in parentheses for the four tree species (*A. negundo* (ACNE), *P. tremuloides* (POTR), *L. tulipifera* (LITU) and *S. albidum* (SAAL)) studied and four factors (N fertilization, rooting depth, number of neighbors and season of birth) plus resulting interactions. Season of birth has three risk ratios per species corresponding to the three birth categories used (i.e., April–June, July–September and after September). Significant results ($P \leq 0.05$) are in bold.

	ACNE	POTR	LITU	SAAL
N fertilization	**0.0256** (0.73)	**<0.0001** (0.53)	0.2145 (0.69)	0.8608 (0.96)
Rooting depth	**0.0131** (0.97)	**<0.0001** (0.92)	0.0791 (0.95)	0.0573 (0.94)
No. of neighboring roots	**0.0060** (1.06)	**0.0133** (1.01)	0.2534 (0.82)	0.2478 (1.12)
Season of birth	0.2500 (0.94, 1.36, 1.44)	**<0.0001** (0.60, 0.21, 0.34)	0.7175 (1.14, 1.90, 1.66)	0.3117 (0.69, 1.13, 1.64)
N fertilization × rooting depth	0.6056	0.5542	0.0987	0.0593
N fertilization × no. of neighboring roots	0.1375	**0.0007**	0.9337	0.4182
N fertilization × season of birth	**0.0088**	**0.0010**	0.6714	0.9284

root observed through the minirhizotron tube. The number of neighboring roots refers to the number of additional roots observed in the same viewing pane of the minirhizotron tube. Season of birth refers to a categorical assignment based on the birth date of a root, where roots born between April and June of any year were assigned to one category, roots born between July and September were assigned to a second category and roots born after September were assigned to a third category.

Differences in cumulative root length from the in-growth core samples were determined by conducting a two-way analysis of variance (ANOVA) comparing the cumulative root length produced on the final minirhizotron image session across blocks (Figure 2). Two-way ANOVAs with interactions were run to determine differences in root respiration, root length and root N : C between species and treatment (N fertilization or H_2O) (Figure 3). Results were considered statistically significant at $P \leq 0.05$.

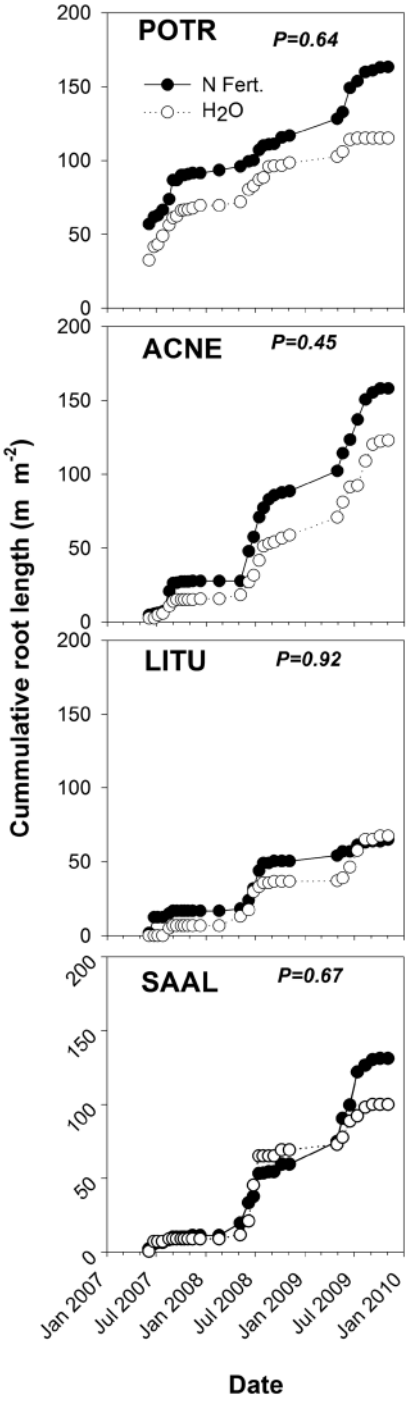

Figure 2. Cumulative root length production assessed with minirhizotrons over 3 years, and of four tree species that varied widely in root morphology. The N fertilization treatment (N Fert., filled circles) was three times soil solution N, with the control (H_2O, open circles) not containing additional N. *P* values obtained from differences across blocks in the cumulative root length produced on the final minirhizotron image session. Tree species were *A. negundo* (ACNE), *P. tremuloides* (POTR), *L. tulipifera* (LITU) and *S. albidum* (SAAL).

Results

Root lifespan was significantly increased by N fertilization in the two fine-root species, *P. tremuloides* (POTR, $P = <0.0001$, first-

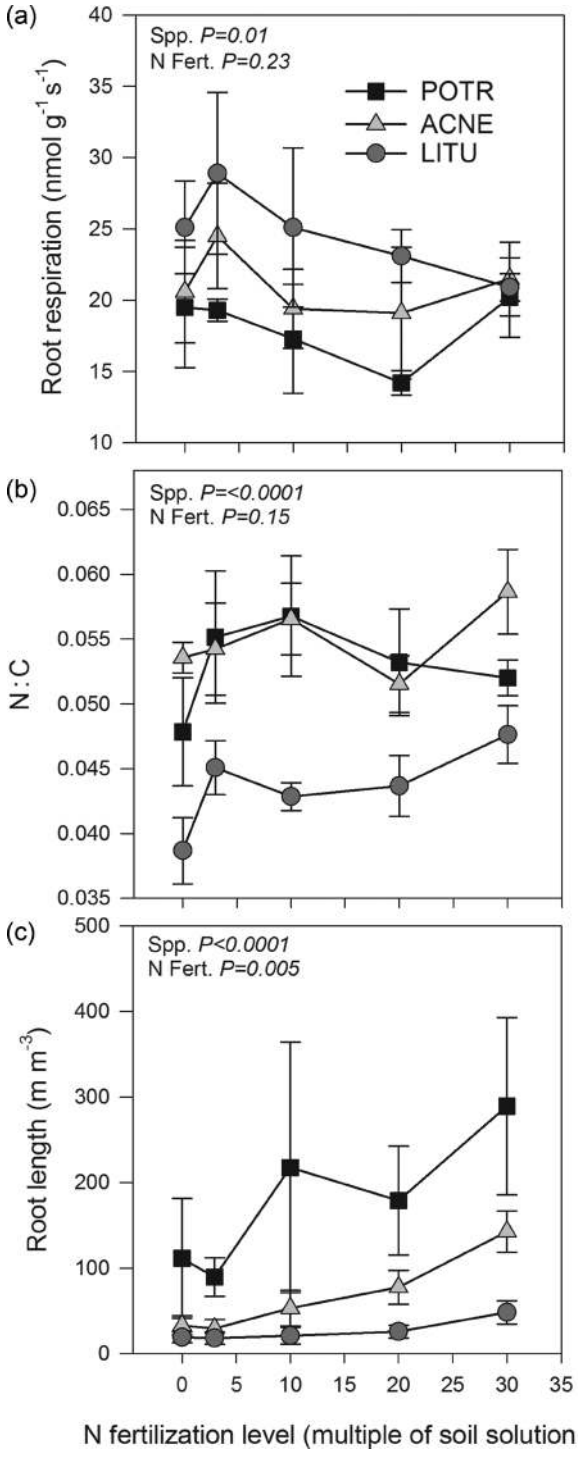

Figure 3. Effects of multiple levels of N addition on root respiration, N : C ratio and root length from in-growth cores of three tree species that vary widely in root morphology. Tree species denoted as: POTR, squares; ACNE, triangles and LITU, circles. (a) The relationship between N fertilization level (as a multiple of soil solution N) and root respiration (species effect: $P = 0.01$; N fertilization effect: $P = 0.23$). (b) The relationship between N fertilization level, as a multiple of soil solution N and root N : C (species effect: $P < 0.0001$; N fertilization effect: $P = 0.15$). (c) The relationship between N fertilization level and root length, which was calculated from root mass using species-specific, first-order SRL values (species effect: $P < 0.0001$; N fertilization effect: $P = 0.005$). Error bars denote standard errors.

order root SRL = 65.2 ± 2.1 m g^{-1}, diameter = 0.17 ± 0.003 mm) and *A. negundo* (ACNE $P = 0.0009$, first-order root SRL = 44.5 ± 2.7 m g^{-1}, diameter = 0.23 ± 0.007 mm), but had no significant effect on the lifespan of the two coarse-root species, *L. tulipifera* (LITU, $P = 0.14$, first-order root SRL = 8.9 ± 1.3 m g^{-1}, diameter = 0.67 ± 0.01 mm) and *S. albidum* (SAAL, $P = 0.79$, first-order root SRL = 13.8 ± 0.9 m g^{-1}, diameter = 0.54 ± 0.05 mm, Figure 1). However, the non-significant result of localized N fertilization on LITU root lifespan may be related to the reduced statistical power compared with the fine-root species, because of the smaller number of roots observed for LITU. The median root lifespan of the fertilized fine-root species increased by 48%, from 69 to 102 days, for POTR and by 40%, from 113 to 188 days, for ACNE. The median root lifespan of the coarse-root species for water control and fertilized roots was 284 and 309 days for LITU (9% increase), and 304 and 310 days (2% increase) for SAAL ($P = 0.14$ and 0.79, respectively).

The effect of N fertilization on root production was assessed in two ways: by direct observation of the fertilized and unfertilized minirhizotron tubes and by measuring the total root mass of roots in the in-growth cores receiving the five levels (0 through 30 times background N) of N fertilization and converting root mass to root length. Nitrogen fertilization did not significantly affect cumulative root length production observed using the minirhizotron tubes (Figure 2: N fertilization effect: $P = 0.35$; species effect: $P = 0.28$, species × N fertilization interaction: $P = 0.94$). We also examined root growth responses using in-growth cores (Figure 3c). In-growth root mass was converted to length using species-specific, first-order-root SRL values, assuming that first-order roots accounted for 50% of the total root mass in the in-growth cores, which corresponded reasonably well with observations of the dissected root samples. Localized N fertilization caused a significant increase in the root length ($P = 0.0052$), with fertilization levels of 30 times soil solution N resulting in 2.5 times more root length in LITU, three times more root length in POTR and almost five times more root length in ACNE compared with zero or three times solution N (no data available for SAAL). Tree species differed in in-growth core root length consistently across all N levels, with LITU having the shortest length and POTR having the longest (species effect: $P < 0.0001$). In all three species, there was no significant difference in in-growth core root length between zero and three times N fertilization, which was consistent with the lack of significance in cumulative root length production observed in our minirhizotron tubes (Figure 2).

Contrary to expectations, N fertilization did not affect the N : C ratio (used as a proxy for N concentration because N : C ratio avoids errors in dry weight estimation from soil contamination on the root surfaces) of the first- and second-order roots from the in-growth cores (Figure 3b: N fertilization effect: $P = 0.15$). However, the root N : C ratio differed between tree species, with the lowest N : C ratio in LITU and the highest in ACNE (species effect: $P < 0.0001$).

Nitrogen fertilization did not significantly increase root respiration across species (Figure 3a: N fertilization effect: $P = 0.22$). In addition, no significant correlation was found between root N : C and root respiration across all treatments ($P = 0.54$). As with root N : C, there were significant species differences in respiration, with LITU exhibiting the fastest root respiration and POTR exhibiting the slowest (Figure 3a; species effect: $P = 0.01$).

In addition to examining the main effect of localized N fertilization on root lifespan, we also examined the effect of rooting depth, number of neighboring roots, season of birth and any resulting interactions on root lifespan using Cox proportional hazard analyses (Table 1). The root diameter was not included in this analysis because our strict diameter criteria (see Materials and methods) for roots to be included in our data set precluded the inclusion of a wide range of root diameters. Rooting depth only significantly affected root lifespan for two of the four study species (ACNE and POTR), with roots living deeper in the soil having a decreased risk of death and therefore living longer (i.e., having a hazard ratio of <1). The lack of a consistent effect of rooting depth on root lifespan across all species studied may be an artifact of the relatively short minirhizotron tubes (i.e., 45 cm, allowing for root observations of the upper 20 cm of soil) used in this study. The number of neighboring roots also significantly affected lifespan for two of the four species investigated (ACNE and POTR), with roots living with more neighbors having shorter lifespans. These species also had the highest root density observed through the minirhizotron tubes. As such, competition among neighboring roots may negatively affect lifespan at high root densities. The seasonality of root birth significantly affected root lifespan for one of the four species investigated (POTR), with roots born later in the season having longer lifespans than those born earlier in the season.

In addition to the main effects on root lifespan, there was a significant interaction between localized N fertilization and the season of root birth in both fine-root species (ACNE and POTR, Table 1). For ACNE, fertilized roots had significantly longer lifespans when born early (April–June) or late (after September) in the season but not over the summer (July–September). For POTR, fertilized roots had significantly longer lifespans when born during the spring (April–June) and the summer (July–September), but not later in the season (after September). For POTR, there was also a significant interaction between N fertilization and the number of neighbors. The number of neighboring roots only significantly affected survivorship when one to three, or greater than six, neighboring roots were present, but not when zero or four to six neighbors were present. In general, although competition from neighboring roots may interact with the N fertilization treatments

resulting in a significant interaction, we are unsure how the exact number of neighboring roots causes significant differences in this interaction.

Discussion

In this common garden study, we created sustained, localized, N-rich patches in which root lifespans could be observed and root samples could be collected to estimate shifts in N concentration and respiration. We found that the root lifespan of the fine-root species was significantly increased by localized N fertilization, whereas the root lifespan of the coarse-root species was either virtually unchanged by N fertilization (SAAL) or possibly modestly increased, for which we lacked the statistical power to detect (LITU) (Figure 1). Surprisingly, we observed no increases in root length in response to a threefold increase in localized N fertilization, but did observe root length increases at higher levels of N fertilization (Figure 3c). In addition, we found that neither root N : C nor root respiration increased with N addition (Figure 3a and b).

Assuming resource optimization, we hypothesized that root lifespan should maximize the lifetime root efficiency or the lifetime benefits relative to the lifetime costs (Yanai et al. 1995). Therefore, roots growing in the N-rich patches that do not become depleted should be longer lived, because they presumably are supplying more of the limiting resource, N, than roots elsewhere on the tree that are foraging in less-fertile soil. Additionally, resource optimization predicts that species with coarse, low-SRL roots should have longer root lifespans, because these roots are more costly, in terms of C to construct for the deployment of length or surface area (Yanai et al. 1995). In both cases, we found support for the resource optimization hypothesis. Species with coarse, low-SRL roots had longer median lifespans than those of high SRL (Figure 4), and high-SRL species had significantly longer median lifespans in N-fertilized versus unfertilized patches (Figure 1). At the same time, the cost of maintaining these roots, as measured by respiration, was not significantly altered by fertilization (Figure 3a). However, because the result of increased root lifespan with N fertilization was not seen across all species, additional factors beyond rapid adjustments in root system resource optimization might be influencing root lifespan in some species.

Our results suggest that a key difference in root lifespan between species in response to N fertilization is a result of variation in plasticity of the physiological traits that control root lifespan. High-SRL species clearly responded to localized N fertilization by extending their lifespan, in contrast to the low-SRL species, where we saw no response in one species (SAAL) and a relatively small, non-significant response in the other species (LITU) (Figure 1). This plasticity of the fine-root species may offset the limitations of their shorter median root lifespans, thereby conferring an increased ability to utilize

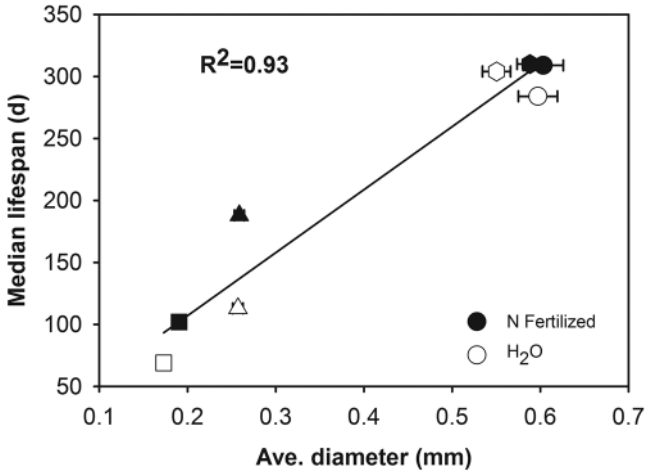

Figure 4. Relationship between median root lifespan and root diameter of four tree species that varied widely in root morphology. Tree species denoted as: POTR, squares, ACNE, triangles, LITU, circles and SAAL, hexagons. The N fertilization treatment (N Fertilized, closed circles) was three times soil solution N, with the control (H_2O, open circles) not containing additional N. Regression line equation: $y = 508.43x + 6.02$. Error bars denote standard errors.

resource heterogeneity. Based on these findings, there appears to be clear species-specific differences in plasticity in root lifespan. The mechanisms behind these observed differences in root lifespan plasticity were not directly studied here, but may arise from differences in the ability to mobilize defense compounds, differences in colonization of mycorrhizal fungal symbionts or differences in the ability to scavenge reactive oxygen and reactive N species.

Although plasticity in plant traits has been studied in aboveground structures, relatively little experimental work has been conducted examining root plasticity under field conditions (see Hodge 2004, Hodge et al. 2009) and, to our knowledge, no studies have examined the relationships among root lifespan, diameter, plasticity and localized nutrient availability. Our results suggest that a tradeoff may exist between phylogenetically constrained root morphology (Comas and Eissenstat 2009, Chen et al. 2013) and root plasticity. Although we did not set out to test the positive relationship between species growth rate and root plasticity (Grime 1977), in our study system it does not appear that the root plasticity that we observed is correlated with species growth rate, because all of the species examined have high relative growth rates. In fact, LITU is one of the fastest growing trees in the common garden in terms of height and trunk diameter, yet the lifespan responses of LITU roots to fertilization were relatively modest and not statistically significant. Additionally, because the tree species investigated co-occur naturally and are known to inhabit moderately fertile soils, the differences in root morphology and the corresponding differences in root lifespan plasticity were not related to adaptations to different soil fertility levels.

Root proliferation in response to N fertilization was generally low in this study and relative responses were fairly similar across species. Localized N fertilization rates at three times that of the high range of naturally occurring available soil solution N caused no enhanced proliferation in either the in-growth cores or minirhizotrons, despite the strong lifespan responses in the fine-root species. At higher rates of N addition in the in-growth core portion of our study, we observed an increase in root length density for all tree species, with a non-significant tendency for fine-root species to exhibit slightly more proliferation than coarse-root species ($P = 0.40$). The greater root proliferation in disturbed soil of fine-root species compared with coarse-root species had previously been observed in a common garden study of citrus roots with a common shoot cultivar (Eissenstat 1991); however, the influence of N fertilization was not examined. In a study in a mixed forest stand near our common garden experiment, fine-root species had greater root proliferation in disturbed soil patches compared with coarse-root species, but again fine-root species only showed a tendency of greater root proliferation to fertilization than coarse-root species (D.M. Eissenstat et al., unpublished data; $P = 0.19$). Collectively, these field studies suggest that although root morphology may influence plasticity in root proliferation in response to N fertilization, the response is generally weak compared with the natural variability in proliferation.

In conclusion, our study clearly indicates that plant species vary in root lifespan responses to nutrient-rich patches. We found that root lifespan was clearly increased by N-rich patches in tree species with fine-root morphology and was negligibly increased in one of the two species with coarse-root morphology. Additionally, the low-SRL species, whose roots are more costly to construct, in general, had longer lifespan than high-SRL species. Additional studies are needed to confirm the potential linkages of root morphology with root proliferation and root lifespan.

Acknowledgments

We thank Travis Haussner and Matt Towland for their help in collecting and processing the minirhizotron images.

Conflict of interest

None declared.

Funding

This work was supported by grants from the U.S. National Science Foundation (OEI-0613832 and IOS-1120482) to D.M.E. Funding to pay the Open Access publication charges for this article was provided by National Science Foundation.

References

Bai WM, Wang ZW, Chen QS, Zhang WH, Li LH (2008) Spatial and temporal effects of nitrogen addition on root life span of *Leymus chinensis* in a typical steppe of Inner Mongolia. Funct Ecol 22:583–591.

Bloom AJ, Sukrapanna SS, Warner RL (1992) Root respiration associated with ammonium and nitrate absorption and assimilation by barley. Plant Physiol 99:1294–1301.

Bouma TJ, Broekhuysen AGM, Veen BW (1996) Analysis of root respiration of *Solanum tuberosum* as related to growth, ion uptake, and maintenance of biomass. Plant Physiol Biochem 34:795–806.

Chen W, Zeng H, Eissenstat DM, Guo D (2013) Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. Glob Ecol Biogeogr 22:846–856.

Comas LH, Eissenstat DM (2009) Patterns in root trait variation among 25 co-existing North American forest species. New Phytol 182:919–928.

Cox DR (1972) Regression models and life-tables. J Roy Stat Soc B 34: 187–220.

Eissenstat DM (1991) On the relationship between specific root length and the rate of root proliferation – a field study using citrus rootstocks. New Phytol 118:63–68.

Eissenstat DM, Caldwell MM (1988) Seasonal timing of root growth in favorable microsites. Ecology 69:870–873.

Eissenstat DM, Yanai RD (1997) The ecology of root lifespan. Adv Ecol Res 27:1–60.

Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL (2000) Building roots in a changing environment: implications for root longevity. New Phytol 147:33–42.

Fitter AH (1994) Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. In: Caldwell MM, Pearcy RW (eds) Exploitation of environmental heterogeneity by plants. Ecophysiological processess above- and belowground. Academic Press, New York, pp 305–324.

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169–1194.

Harpster TL (2011) Evaluating the soil building characteristics and growth benefits of four-composted organic waste products on field grown woody nursery stock. Masters thesis, The Pennsylvania State University, PA, USA.

Hodge A (2004) The plastic root: root responses to heterogeneous supplies of nutrients. New Phytol 162:9–24.

Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil 321:153–187.

Jackson RB, Caldwell MM (1993) Geostatistical patterns of soil heterogeneity around individual perennial plants. J Ecol 81:683–692.

Jackson RB, Mooney HA, Schulze ED (1997) A global budget for fine root biomass, surface area, and nutrient contents. Proc Natl Acad Sci USA 94:7362–7366.

McCormack ML, Adams TS, Smithwick EAH, Eissenstat DM (2012) Predicting fine root lifespan from plant functional traits in temperate trees. New Phytol 195:823–831.

Norby RJ, Jackson RB (2000) Root dynamics and global change: seeking an ecosystem perspective. New Phytol 147:3–12.

O'Brien EE, Brown JS, Moll JD (2007) Roots in space: a spatially explicit model for below-ground competition in plants. Proc R Soc B 274:929–935.

Pregitzer KS, Hendrick RL, Fogel R (1993) The demography of fine roots in response to patches of water and nitrogen. New Phytol 125:575–580.

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL (2002) Fine root architecture of nine North American trees. Ecol Monogr 72:293–309.

Robinson D (1996) Variation, co-ordination and compensation in root systems in relation to soil variability. Plant Soil 187:57–66.

Robinson D, Hodge A, Griffiths BS, Fitter AH (1999) Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proc R Soc B 266:431–435.

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115.

Wilson JB (1988) Shoot competition and root competition. J Appl Ecol 25:279–296.

Yanai RD, Fahey TJ, Miller L (1995) Efficiency of nutrient acquisition by fine roots and mycorrhizae. In: Smith W, Hinckley TM (eds) Resource physiology of conifers. Academic Press, New York, pp 75–103.

Zadworny M, Eissenstat DM (2011) Contrasting the morphology, anatomy and fungal colonization of new pioneer and fibrous roots. New Phytol 190:213–221.